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Metabolism and Excretion of 2-Ethoxyethanol in the Adult Male Rat

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The routes of ^{14}C excretion following the administration of a single oral 230 mg/kg body weight dose of 2-ethoxyethanol [ethanol-1,2-^{14}C] or 2-ethoxyethanol [ethoxy-1-^{14}C] to male Sprague-Dawley rats were investigated. Elimination of the ^{14}C by the urinary route accounted for 76 to 80% of the dose within 96 hr. The main pathway of biotransformation is oxidation to the corresponding acid, with some subsequent conjugation of the acid metabolite with glycine. The major metabolites, ethoxyacetic acid and *N*-ethoxyacetyl glycine, representing 73 to 76% of the administered dose, were eliminated in the urine. The major difference in the metabolic profiles of the two radiochemicals was in the rate and amount of $^{14}CO_2$ expired via the lung. Of the administered ^{14}C, 11.7% of the ethoxy-labeled and 4.6% of the ethanol-labeled compounds were eliminated as CO_2. The biological half-time was 9.9 ± 1.5 hr for the ethoxy-labeled compound and 12.5 ± 1.9 hr for the ethanol label. After administration of the ethanol-labeled compound, the only radiolabeled component found in the rat testes was identified as ethoxyacetic acid. Results of this study suggest that the reported testicular effects in the rat may be a result of tissue levels of ethoxyacetic acid.

Introduction

2-Ethoxyethanol is the highest volume ethylene oxide-based glycol ether produced in the United States (*1*). The chemical has been of commercial importance for more than 50 years, being used primarily as a solvent and as an intermediate in the production of 2-ethoxyethyl acetate.

No chronic health effects resulting from industrial use of 2-ethoxyethanol have been reported. However, effects of 2-ethoxyethanol in animals include tubular degeneration in the kidney (*2*), erythrocyte hemolysis (*3,4*), and reduced leukocyte count (*5*). More recently, Nelson et al. (*6*) showed neurochemical and behavioral changes in the offspring of female rats exposed to 2-ethoxyethanol.

Although no adverse reproductive effects resulting from human exposure to 2-ethoxyethanol have been reported, testicular damage in rats exposed to the chemical was described as early as 1942. In that year, Morris et al. (*7*) showed that rats developed tubular atrophy and edema of the testes after the oral administration of 2-ethoxyethanol. Similar effects have also been noted in mice (*5,8*). Miller and his co-workers have demonstrated that 2-methoxyethanol, a closely related glycol ether, causes degeneration of the germinal epithelium of the testes of rats (*9*). Subsequently, Miller et al. (*10*) showed that a similar condition resulted from the administration of methoxyacetic acid, a metabolite of 2-methoxyethanol (*11*). The metabolism of two other ethylene glycol monoalkyl ethers, *n*-butoxyethanol (*12,13*) and isopropoxyethanol (*14*), has been reported to proceed by oxidation to the corresponding carboxylic acid. Conjugation of isopropoxyethanol (*14*) and 2-ethoxyethanol (*15*) metabolites with glycine has been reported. In a previous study of the biotransformation of 2-ethoxyethanol in the rat, Jönsson et al. (*15*) identified ethoxyacetic acid and *N*-ethoxyacetyl glycine as urinary metabolites. These investigators, however, recovered only an estimated 30% of the administered dose.

The objectives of this study were to supplement the earlier work on the biotransformation of 2-ethoxyethanol in the rat by determining the fate of the compound following oral administration and to identify the chemical species associated with testicular damage.

Methods

Chemicals and Dosing Solutions

The test compound, 2-ethoxyethanol (99%), was purchased from Aldrich Chemical Company, Milwaukee,

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WI. Both 2-ethoxyethanol [ethanol-1,2-^{14}C] and 2-ethoxyethanol [ethoxy-1-^{14}C] with specific activities of 3.47 mCi/mmole and 2.69 mCi/mmole, respectively, were purchased from New England Nuclear, Boston, MA. Subsequent purification and analysis of these radiochemicals by high-performance liquid chromatography (HPLC) resulted in a radiochemical purity of 99.6% for each compound. The reference compound, ethoxyacetic acid (98%), was purchased from Aldrich Chemical Company. Ethoxyacetyl chloride (b.p. 124°C, uncorr.) was synthesized in accordance with the general method of Freed (*16*). *N*-Ethoxyacetyl glycine ethyl ester (b.p. 107.5°C/0.01 mm, uncorr.) was synthesized by reaction of ethoxyacetyl chloride with glycine ethyl ester using the method of Wolf and Niemann (*17*).

Treatment solutions were prepared by dissolving 2-ethoxyethanol [ethanol-1,2-^{14}C] or 2-ethoxyethanol [ethoxy-1-^{14}C] along with ethoxyethanol in distilled water to yield a concentration of 46 mg/mL with specific activities of 0.123 μCi/mg and 0.117 μCi/mg, respectively.

Animals, Doses and Collections

Male Sprague-Dawley [Crl:CD (SD)BR outbred], cesarean-derived rats weighing 51 to 75 g, were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) and maintained on NIH-07 rat and mouse diet (Ziegler Brothers, Inc., Gardners, PA) and tap water *ad libitum*. After a 2-week quarantine period, 70 rats, weighing 190 to 210 g, were used in a preliminary study to determine the acute 14-day oral LD_{50} of 2-ethoxyethanol.

The dose range for the toxicity study was established by treating groups of two rats with various amounts of undiluted 2-ethoxyethanol. Upon initiation of the toxicity experiment, the rats were starved overnight, weighed (192.6 ± 12.3), and randomly assigned to six groups of ten rats each. Doses of 250, 500, 1000, 2000, 4000, and 8000 mg/kg body weight were administered, by gavage, to all rats within the corresponding dosage groups. These animals were observed for mortality during the subsequent 14-day period. The LD_{50} value was calculated from the mortality in the several groups by the probit method of Finney (*18*).

In subsequent excretion and metabolism studies, animals in the same weight range were transferred to individual Roth-type glass metabolism cages. Laboratory air, dried and freed of carbon dioxide, organics, and particulates, was passed through the metabolic chambers at a flow rate of 0.5 L/min. Except for an 18-hr starvation period immediately prior to dosing, the animals had free access to food sticks formed by baking ground NIH-07 diet with a raw egg binder. Distilled water was available throughout the experimental period. Following acclimatization to the cages for 3 days prior to dosing, animals were given a single oral 230 mg/kg body weight dose of either 2-ethoxyethanol [ethanol-1,2-^{14}C] or 2-ethoxyethanol [ethoxy-1-^{14}C]. This dose corresponds to 0.1 of the LD_{50} value determined in the preliminary experiment. Air exiting each chamber was passed first through a sorbent sampling tube consisting of a 400-mg front section and a 200-mg back section of activated charcoal (SKC Inc., Eighty Four, PA) for collection of organic material appearing in the expired air and then bubbled through a tower containing ethanolamine to trap expired carbon dioxide. Sampling tubes and ethanolamine solution in the towers were changed periodically as required to avoid loss of radioactivity. Urine was collected when voided for the first 30 hr, and at 48, 72, and 96 hr after dosing. Urine was diluted to 10 or 25 mL and stored at -20°C until time of analysis. Feces were collected at 24-hr intervals and also stored at -20°C. At the end of the 96-hr experimental period, the animals were killed by asphyxiation with CO_2. Residual radioactivity in the metabolism cages was collected by washing first with water and then with methanol.

In a separate experiment, six animals, weighing 190 to 210 g, were starved for an 18-hr period and given a single oral 230 mg/kg body weight dose of 2-ethoxyethanol [ethanol-1,2-^{14}C]. Animals were killed by decapitation and exsanguination at 1 hr intervals after dosing. Testes were excised and stored at -20°C until time of analysis.

Scintillation Spectrometry

Urine samples were prepared for ^{14}C analysis by dissolving duplicate 0.1-mL aliquots in 3 mL of methanol and 10 mL of scintillator consisting of 5.5 g Permablend I (Packard Instrument Co., Downers Grove, IL) per liter of toluene. Duplicate 1-mL aliquots of ethanolamine solution were prepared for counting in the same scintillator. Feces samples were ground frozen with microcrystalline cellulose (E. Merck, Darmstadt, F.R.G.) using a Model A10 analytical mill (Tekmar Company, Cincinnati, OH). Five portions of each milled feces sample were prepared for ^{14}C analysis by combustion in a Tri-Carb Model B306 sample oxidizer (Packard Instrument Co.). Front and back sections of charcoal from sample collection tubes were combusted separately in the sample oxidizer. Carcasses were solubilized in 1 N NaOH prepared in 70% (v/v) aqueous methanol, and five portions of the digest were combusted. Cage washings were homogenized using a Polytron Model PCU-1 homogenizer equipped with a PC-10 probe (Kinematica Gmbh, Luzern, Switzerland), and five 0.5-mL aliquots were combusted. Carbon-14 was determined using a Model LS8100 liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, CA), and data were corrected for background and quenching. Counting efficiency was calculated using an external standard.

Separation and Isolation of Metabolites

Urinary metabolites were separated by HPLC with a 50 cm × 9 mm internal diameter stainless steel column

packed with Partisil-10 OD-2 (Whatman, Inc., Clifton, NJ). A model ALC/GPC 201 high-performance liquid chromatograph equipped with two Model 6000A pumps and a Model U6K injector (Waters Associates, Inc., Milford, MA) was used throughout the study. Solvent gradient conditions were controlled by a Model 720 system controller (Waters Associates, Inc.). Individual urine samples were thawed, adjusted to pH 3 with glacial acetic acid, and filtered through a 0.45-μm pore size disposable filter assembly (Acrodisk, Gelman Sciences, Ann Arbor, MI) prior to HPLC analysis. A portion of each of these samples was initially chromatographed for 20 min isocratically with 1% acetic acid in water. Metabolites were then eluted by using a methanol:1% acetic acid solvent system, linearly programmed from 15% to 80% methanol in 40 min. The solvent flow rate was maintained at 1 mL/min during the 60-min period of analysis. Radioactive metabolites were detected and quantified by using an in-line Tri-Carb RAM 7500 radioactivity monitor equipped with a quartz flow cell packed with a solid scintillator (Packard Instrument Co.).

Another portion of urine was acidified to pH 1 with 6 N HCl and heated at 90°C for 4 hr under nitrogen to hydrolyze possible conjugates. The urine was then adjusted to pH 3 with 6 N NaOH, filtered, and analyzed by HPLC.

Urinary metabolites were collected, pooled by retention time, and lyophilized using a Model 75035 freeze dryer (Labconco Corporation, Kansas City, MO). Residues were subsequently taken up in 1 mL of methanol for identification of metabolites by chromatographic techniques.

Testes were also examined for the presence of radioactive compounds by HPLC. Rat testes were thawed, minced, and homogenized in three volumes of distilled water using a Polytron Model PCU-1 homogenizer equipped with a PC-10 probe. Homogenates were lyophilized, and the residues were extracted three times with 1-mL volumes of methanol. These extracts were combined and concentrated to 1 mL for HPLC analysis using the same chromatographic conditions described previously. The single radioactive fraction was collected for further characterization by gas–liquid chromatography (GLC) and thin-layer chromatography (TLC).

Characterization of Metabolites

Retention times of metabolites isolated from the urine and testes and of reference compounds were determined on two different gas chromatographic columns. Initially the metabolites were individually chromatographed on a 183 cm × 2 mm glass column packed with 10% SP-2100 on 100/120 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) installed in a Model 3920B gas chromatograph (Perkin-Elmer Corp., Norwalk, CT). The oven temperature was programmed from 50 to 250°C at 2°C/min, with a nitrogen carrier gas flow rate of 30 cm^3/min. Metabolites found to be nonvolatile were esterified by using the general method of Thenot et al. (*19*) and rechromatographed. Metabolites were also chromatographed on a 183 cm × 2 mm glass column packed with 10% SP-1200/1% H_3PO_4 on 80/100 mesh Chromosorb W AW (Supelco, Inc.), a column useful for the separation of organic acids. The oven temperature was programmed from 70 to 200°C at 2°C/min. A nitrogen carrier gas flow rate of 30 cm^3/min was used.

Metabolites were further characterized by TLC R_f values. Radioactive metabolites were co-chromatographed with reference compounds on silica-gel precoated glass plates (E. Merck, 60 F 254, 0.25 mm layer thickness). Chromatograms were developed with ethyl acetate:acetic acid:water (90:5:5, v/v). Areas corresponding to radioactive metabolites were located by using a Model 7230 radiochromatograph x-y scanner (Packard Instrument Co.) with a collimator opening of 1.5 mm^2 and a helium:isobutane (99.05:0.95, v/v) counting gas flow rate of 5 cm^3/min. Reference compounds were located by observing the quenching of fluorescence activated by 254 nm radiation and by color reactions with either iodine vapor or 0.05% Bromocresol Green reagent (E. Merck).

The two major urinary metabolites or their ethyl esters were further characterized by determination of boiling points at atmospheric or reduced pressure. In addition, mass spectra of the major urinary metabolite and the corresponding reference compound were obtained by using a Model 5982A mass spectrometer (Hewlett-Packard Co., Palo Alto, CA) operating with an electron energy of 70 eV. A model 5711 gas chromatograph (Hewlett-Packard Co., Avondale, PA), fitted with a 91 cm × 2 mm glass column packed with 3% SP-2300 on 100/120-mesh Supelcoport (Supelco, Inc.), was interfaced to the mass spectrometer through a glass-jet separator. The oven temperature was programmed from 100 to 150°C at 2°C/min. A helium carrier gas flow rate of 30 cm^3/min was used. All mass spectral data were acquired and processed using a Model 5834A data system (Hewlett-Packard Co.).

Statistical Analysis

Statistical differences between groups were determined using Student's t-test. The biological half-times of the two radioisotopes were calculated by linear regression analysis.

Results

Determination of Acute Toxicity

The single-dose oral LD_{50} for 2-ethoxyethanol determined in 190 to 210 g male Sprague-Dawley rats, based upon 14-day mortality data, was determined to be 2300 mg/kg body weight.

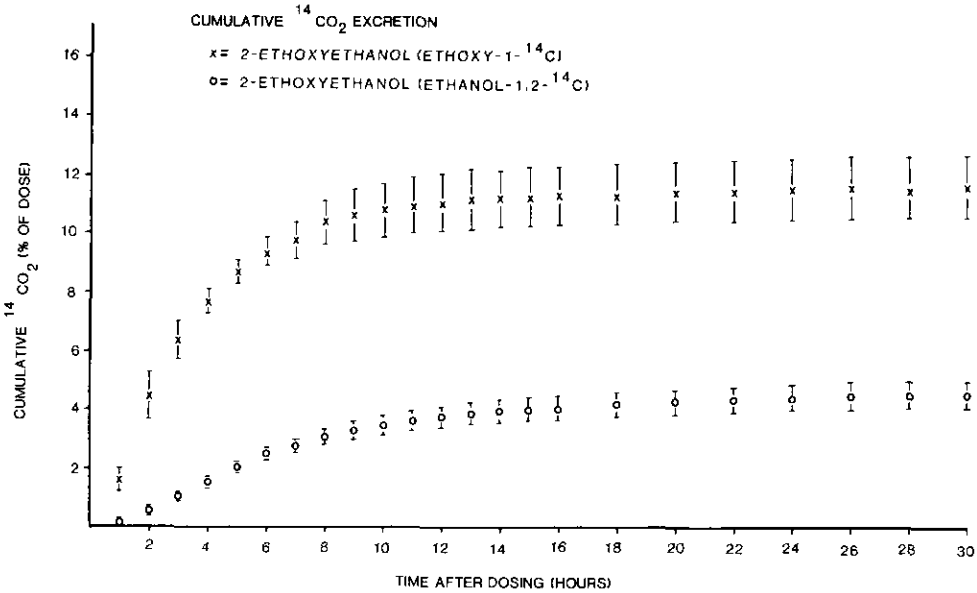

FIGURE 1. Cumulative 30-hr $^{14}CO_2$ excretion after oral administration of 230 mg/kg doses of radiolabeled 2-ethoxyethanol to the adult male rat (mean ± SE).

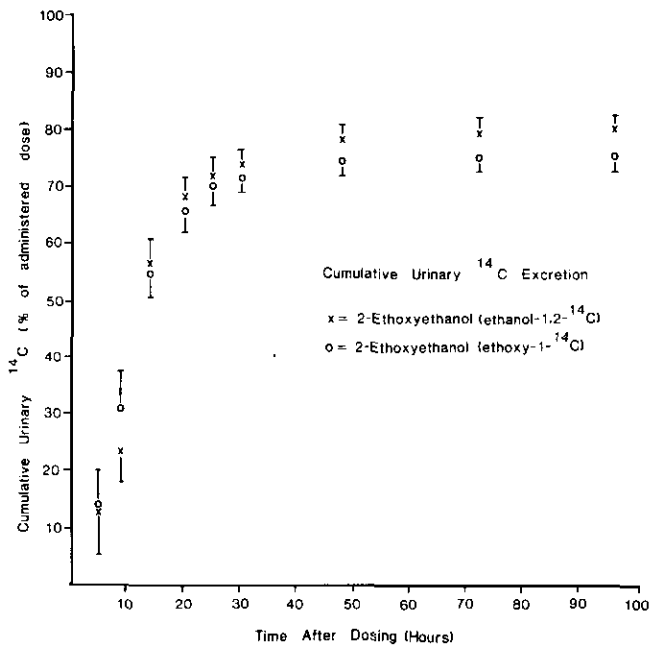

FIGURE 2. Cumulative 96-hr urinary ^{14}C excretion after oral administration of 230 mg/kg doses of radiolabeled 2-ethoxyethanol to the adult male rat (mean ± SE).

Excretion of Radioactivity

The principal route of excretion of the administered radioactivity was via the kidneys. Rats treated with the ethanol-labeled material excreted 80.6% of the dose in the 96-hr collection period. The corresponding value for rats treated with the ethoxy-labeled compound was 75.5% of the dose. When the amounts of ^{14}C in the cage washes—considered to be residual urine—were added,

Table 1. Total 96-hr recovery of ^{14}C.

	% of administered 2-ethoxyethanol	
Sample	[Ethanol-1,2-^{14}C][a]	[Ethoxy-1-^{14}C][b]
Urine	80.6 ± 2.2	75.6 ± 2.1
CO_2	4.6 ± 0.5[c]	11.7 ± 1.0[c*]
Volatile organics	0.2 ± 0.0[c]	0.4 ± 0.0[c]
Feces	4.5 ± 1.2	2.7 ± 0.7
Carcass	4.6 ± 0.2*	1.8 ± 0.2
Cage Wash	4.8 ± 1.9	2.2 ± 0.8
Total recovery	99.3 ± 6.0	94.4 ± 4.8

[a]Animals received 230 mg/kg doses of 2-ethoxyethanol [ethanol-1,2-^{14}C] (specific activity = 0.123 μCi/mg; mean ± SE, n = 4)
[b]Animals received 230 mg/kg doses of 2-ethoxyethanol [ethoxy-1-^{14}C] (specific activity = 0.117 μCi/mg; mean ± SE, n = 5).
[c]30-hr value.
*Statistically different from corresponding value by Student's t test, $p < 0.01$.

the total urinary recoveries became 85.4% and 77.8% of the dose for the ethanol-labeled and ethoxy-labeled compounds, respectively. The elimination of radioactivity in the urine was rapid with 71.6% of the ethanol-label and 69.9% of the ethoxy-label excreted over the first 24 hr. Only an additional 6 to 9% of the administered radioactivity was excreted in the urine during the ensuing 72 hours (Fig. 1). Administration of the ethoxy-labeled compound resulted in the excretion of 11.7% of the dose as respiratory $^{14}CO_2$. In contrast, the ethanol-labeled compound gave rise to only 4.6% of the dose as $^{14}CO_2$. After the first 10 hr, the output of labeled CO_2 rapidly declined. By 30 hr, the expiration of $^{14}CO_2$ had virtually ceased (Fig. 2), and sampling was discontinued at that time. Only trace amounts of volatile organics were found in the breath of rats treated with either radiolabeled compound. Relatively minor amounts of ^{14}C were excreted in the feces or remained

in the carcass at 96 hr after treatment with either radiochemical. The biological half-times for the ethanol- and ethoxy-labeled compounds were 12.5 ± 1.9 hr and 9.9 ± 1.5 hr, respectively. Total recovery of ^{14}C was 99.3% from rats dosed with 2-ethoxyethanol [ethanol-1,2-^{14}C] and 94.4% from those dosed with 2-ethoxyethanol [ethoxy-1-^{14}C]. These results are summarized in Table 1.

Metabolism of 2-Ethoxyethanol

The urinary metabolites excreted by rats following administration of either radiochemical were separated by HPLC and quantified by scintillation spectrometry. The relative amounts of each metabolite were calculated by summation of these analytical results for each urine sample collected over the 96-hr period (Table 2). Of the 12 distinct radiolabeled components present in the urine of rats treated with either radiochemical, only two minor components were found not to be common metabolites of both. Urinary metabolite E, representing 1.4% of the administered ^{14}C, was specific for the ethanol-labeled compound, whereas metabolite F, representing 1.5%, appeared only in the urine of rats treated with the ethoxy-labeled compound.

The two major metabolites together accounted for 73

Table 2. 96-Hr urinary metabolic profile.

Metabolite	HPLC retention time(Rt), min	[Ethanol-1,2-^{14}C][a]	[Ethoxy-1-^{14}C][b]
A	25.8	< 0.01	< 0.01
B	27.4	0.21 ± 0.02	0.01 ± 0.05
C	28.8	1.76 ± 0.53	0.09 ± 0.01
D	42.6	0.32 ± 0.09	0.18 ± 0.03
E	45.6	1.44 ± 0.41	—
F	47.4	—	1.51 ± 0.23
G	50.2	32.13 ± 2.23	28.04 ± 1.32
H	52.5	43.44 ± 2.09	44.88 ± 2.62
I	53.4	0.25 ± 0.05	0.16 ± 0.05
J	54.0	0.42 ± 0.09	0.27 ± 0.13
K	56.0	0.18 ± 0.03	0.07 ± 0.03
L	58.4	0.10 ± 0.02	0.02 ± 0.01
Total recovery		80.25 ± 5.56	75.32 ± 4.48

[a]Animals received 230 mg/kg doses of 2-ethoxyethanol-1,2-^{14}C] (specific activity = 0.123 μCi/mg; mean ± SE, n = 4).
[b]Animals received 230 mg/kg doses of 2-ethoxyethanol [ethoxy-1-^{14}C] (specific activity = 0.117 μCi/mg; mean ± SE, n = 5).

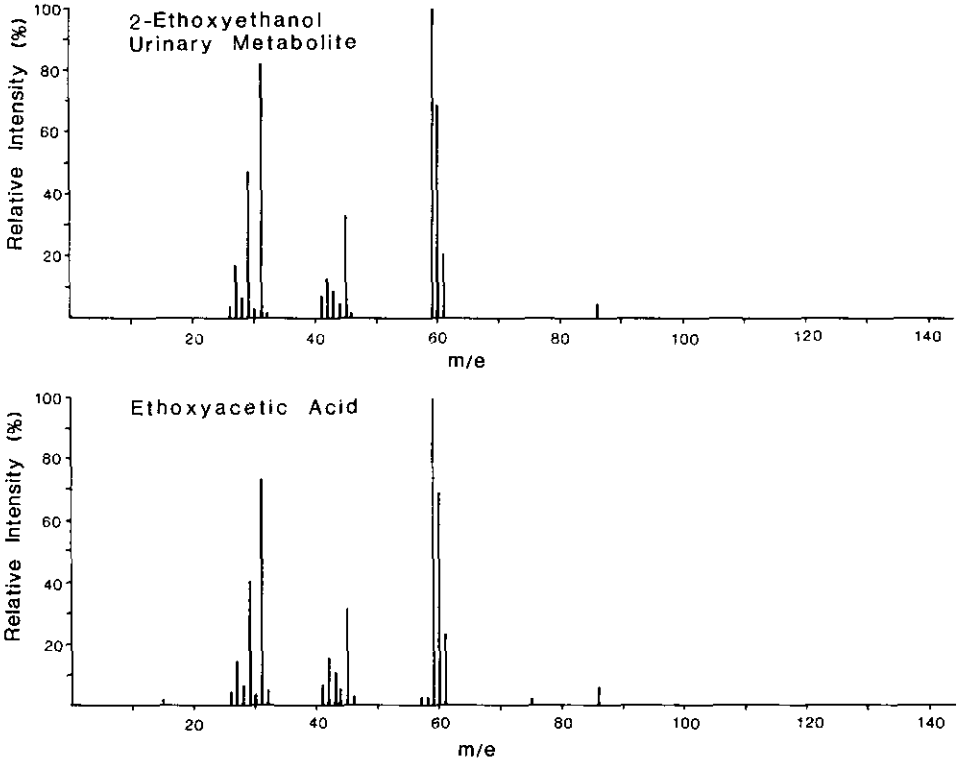

FIGURE 3. Comparison of mass spectra of ethoxyacetic acid and metabolite isolated from rat urine after oral administration of 230 mg/kg doses of 2-ethoxyethanol.

Table 3. Comparison of urinary metabolites of 2-ethoxyethanol with reference compounds.

Compound	Retention time A, min[a]	Retention time B, min[b]	R_f[c]	Boiling point, °C
Ethoxyacetic acid	4.9	23.2	0.33	206
Metabolite H	4.7	23.3	0.32	207
Testicular metabolite	4.5	23.3	0.32	—
N-Ethoxyacetyl glycine, ethyl ester	22.6	47.1	0.25	107.5/0.01 mm
Metabolite G, ethyl ester	23.0	45.3	0.25	107.5/0.01 mm
2-Ethoxyethanol	5.3	2.5	0.54	135.1

[a]GLC retention time (min) using a 183 cm × 2 mm i.d. glass column packed with 10% SP-2100 on 100/120-mesh Supelcoport. Oven temperature programmed from 50° to 250° at 2°C/min with 30 cm^3/min nitrogen carrier gas.

[b]GLC retention time using a 183 cm × 2 mm i.d. glass column packed with 10% SP-1200/1% H_3PO_4 on 80/100-mesh Supelcoport. Oven temperature programmed from 70° to 200°C at 2°C/min with 30 cm^3/min nitrogen carrier gas.

[c]TLC R_f value of compounds spotted on E. Merck Silica gel F 60 precoated plates and run 10 cm with ethyl acetate:acetic acid:water (95:5:5) mobile phase.

to 76% of the administered radioactive dose. The more abundant of these, metabolite H, accounted for 43.4% of the administered ethanol-labeled compound and 44.9% of the ethoxy-labeled compound. The second most abundant, metabolite G, amounted to 32.1% of the administered ethanol-labeled compound and 28.0% for the ethoxy-labeled compound. The remaining eight radioactive components accounted for less than 3% of the administered dose. Analysis by HPLC of urine subjected to acid hydrolysis showed a disappearance of metabolite G with a concomitant increase in metabolite H, indicating the presence of a possible glycine conjugate.

Urinary metabolite H, isolated by HPLC, was further purified by fractional distillation at atmospheric pressure to yield a colorless liquid, boiling at 207°C. This metabolite was subsequently identified as ethoxyacetic acid by comparison of boiling points, GLC retention times on two different columns, and TLC R_f values with those of the authentic reference compound. The identification of this compound was confirmed by mass spectral analysis (Fig. 3).

Urinary metabolite G did not elute from the gas chromatographic column under the conditions used. Subsequently, the ethyl ester of this metabolite was formed and isolated by fractional distillation at reduced pressure to yield a colorless liquid boiling at 107.5°C/0.01 mm Hg. This metabolite was further identified as *N*-ethoxyacetyl glycine by comparison of boiling points, GLC retention times on two different columns, and TLC R_f values of its ethyl ester with those of the authentic compound (Table 3).

Minor metabolites in the urine, accounting for only 3 to 5% of the administered ^{14}C, were not identified. However, their failure to respond to conditions of hydrolysis or esterifiction indicates that these metabolites were not amino acid or glucuronic acid conjugates.

Identification of Metabolites Isolated from Testes

Analysis of extracts of rat testes by HPLC indicated that the amount of ^{14}C in the testes peaked at 2 hr after administration of the ethanol-labeled compound and declined rapidly over the ensuing 4-hr period (Table 4). The single radioactive HPLC peak was subsequently identified as ethoxyacetic acid by comparison of GLC retention times on two different columns, TLC R_f values and HPLC retention times with those of the authentic compound.

Table 4. Testicular ^{14}C levels after administration of 2-ethoxyethanol [ethanol-1,2-^{14}C].

Time after dosing, hr[a]	% of dose
1	0.19
2	0.31
3	0.19
4	0.16
5	0.11
6	0.06

[a]Animals received 230 mg/kg oral doses of 2-ethoxyethanol [ethanol-1,2-^{14}C] (specific activity = 0.117 μCi/mg). Testes of each animal were homogenized in three volumes of distilled water, and lyophilized. Residues were extracted three times with 1-mL portions of methanol and chromatographed by HPLC.

Discussion

The results of this study indicate that metabolism of 2-ethoxyethanol in the rat proceeds primarily through oxidation to the corresponding acid, with some subsequent conjugation of the acid metabolite with glycine. These results confirm the work of Jönsson et al. (*15*) and are consistent with the biotransformation pathways reported for other ethylene glycol monoalkyl ethers (*11–14*). In the present study, the principal difference observed in the metabolic profiles of the two different labeled 2-ethoxyethanols was in the higher amount of expired $^{14}CO_2$ from the ethoxy-labeled compound with a concomitant shortening of the biological half-time. After administration of radiolabeled 2-ethoxyethanol, a single metabolite, ethoxyacetic acid, was identified in the rat testes.

Although the metabolism of 2-ethoxyethanol has been studied to some extent, information pertaining to its quantitative metabolism and excretion has not been previously reported. After incubation of 2-ethoxyethanol with *Acetobacter suboxidans*, Hrotmatka and Polesofsky

Table 5. Metabolism of 2-ethoxyethanol.

Metabolite reported	Species	Reference
Ethoxyacetic acid	*Acetobacter suboxidans*	(*20*)
	Corynebacter sp.	(*21*)
	Alcaligenes sp.	
	Rat	(*15*)
N-Ethoxyacetyl glycine	Rat	(*15*)

(*20*) reported finding ethoxyacetic acid and suggested that this metabolite would be more toxic than the parent compound. Using a similar bacterial system, Harada and Nagashima (*21*) noted that 2-ethoxyethanol was utilized as the sole carbon source and postulated that, following the initial conversion to ethoxyacetic acid, the ether bond was cleaved. Jönsson et al. (15) reported finding two metabolites, ethoxyacetic acid and *N*-ethoxyacetyl glycine, in the urine of rats after oral administration of 2-ethoxyethanol. These investigators, using unlabeled 2-ethoxyethanol, estimated that the combined excretion of the two metabolites amounted to approximately 30% (mole/mole) of the given dose. The results of the studies are summarized in Table 5.

The metabolism of 2-ethoxyethanol appears to be complex as indicated by the number of radiolabeled urinary components found by HPLC. The major metabolites, ethoxyacetic acid and *N*-ethoxyacetyl glycine, account for 73 to 76% of the administered radioactivity. Many of the 10 minor urinary components, representing 3–5% of the dose, may be products of cleavage of the ether linkage. Unchanged 2-ethoxyethanol was not detected in the urine. The 0.2 to 0.4% of the administered ^{14}C collected on charcoal was not further characterized and may represent unchanged 2-ethoxyethanol expired via the lung.

Previous studies of ethylene glycol monoalkyl ethers have noted $^{14}CO_2$ in the expired breath of rats, but no conclusions were drawn regarding possible cleavage of the ether bond. Miller and his co-workers (*11*) found that 12% of administered radioactivity was eliminated as $^{14}CO_2$ in 48 hr using 2-methoxyethanol [ethanol-1,2-^{14}C]. After administration of isopropoxyethanol [ethanol-1,2-^{14}C], Hutson and Pickering (*14*) recovered 16% as $^{14}CO_2$ in 96 hr. Since, in both studies, the terminal carbon was radiolabeled, $^{14}CO_2$ production involving this portion of the molecule could not be ruled out. Biological cleavage of the carbon-oxygen bond in diethyl ether, however, has been reported in the rat (*22*), rabbit (*23*), mouse (*24*) and man (*25,26*). In the present study, cleavage of the ethoxy-labeled compound resulted in the elimination of 11.7% of the dose as $^{14}CO_2$. However, only 4.6% of the administered ethanol-labeled compound was eliminated as $^{14}CO_2$. These results show that the ether linkage of 2-ethoxyethanol was cleaved to the extent of at least 11.7% in the rat.

Although testicular damage has been reported in the rat following administration of certain of the ethylene glycol monoalkyl ethers (7,9), accumulation of the compounds or their metabolites in the testes does not appear likely. Miller et al. (*10*), studying radiolabeled 2-methoxyethanol, found only 0.13% of the orally administered ^{14}C in the testes after 48 hr. In addition, these investigators noted that the amount of ^{14}C in the testes was low in comparison with that in the blood, indicating a lack of accumulation. In the present study, the level of ^{14}C in the testes was highest at 2 hr after the administration of 2-ethoxyethanol [ethanol-1,2-^{14}C] and decreased rapidly thereafter. The only radioactive compound detected in that tissue was ethoxyacetic acid. These results are consistent with the findings of Miller et al. (*11*) for methoxyacetic acid and suggest that the acid metabolites may be the activating agents for the testicular changes resulting from exposure to the ethylene glycol monoalkyl ethers.

Mention of company or product names is not to be considered an endorsement by the National Institute for Occupational Safety and Health.

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